WARS2 mutations cause dopa-responsive early-onset Parkinson's disease and myoclonus dystonia phenotypes

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Abstract

Introduction

To date, only 16 subjects with biallelic *WARS2* gene mutations, encoding the tryptophanyl mitochondrial aminoacyl-tRNA synthetase, presenting with a neonatal or infantile onset mitochondrial disease have been reported. Here, we present additional 6 cases of *WARS2*-related

disease and expand the spectrum of this disorder to later onset phenotypes including a dopa-responsive early-onset Parkinson's disease (PD) and myoclonus-dystonia syndrome.

Methods

Six patients from 4 families underwent whole-exome sequencing within research and diagnostic settings. Following identification of the genetic defects, in-depth phenotyping and protein expression studies were performed.

Results

A relatively common pathogenic p.Trp13Gly missense variant in *WARS2* was detected in all 6 subjects in combination with different other pathogenic alleles (exon 2 deletion in family 1; p.Leu100del in family 2; p.Gly50Asp in family 3; and p.Glu208* in family 4). Two subjects presented with action tremor around age 10-12 years and developed tremor-dominant PD with prominent neuropsychiatric features later in their twenties. Two subjects presented with a myoclonus-dystonia dominant phenotype, whereas the phenotypic spectrum in the remaining 2 subjects was consistent with that of previously reported cases. Western blotting analyses in patient-derived fibroblasts, showed markedly decreased expression of the full-length WARS2 protein in both subjects carrying p.Trp13Gly and a exon-2 deletion in compound heterozygosity.

Discussion

Our study is the first to expand the spectrum of the disease to later onset phenotypes of early-onset tremor-dominant PD and myoclonus dystonia phenotypes. In case of suspected *WARS2*-related disease, relaxing of exome filters might be crucial in order to prioritize the recurrent causative p.Trp13Gly variant.

Keywords: WARS2, early onset Parkinson's disease, myoclonus dystonia, whole exome sequencing

Introduction

Mitochondrial aminoacyl-tRNA synthetases (mt-aRSs) are essential components of the translation process in mitochondria, charging tRNAs with their cognate amino acids during translation of mitochondrial genes (Ibba 2000). Defects in mt-aRSs result in defective intramitochondrial translation, affecting mainly the oxidative phosphorylation system complexes with the largest number of mitochondrial-encoded subunits, i.e. complex I and complex IV. The activity of complex II is normal, or even upregulated, as it is exclusively composed of nuclear-encoded subunits (Diodato 2014, Tolkunova 2000). A total of 19 mt-aRSs have been described and all have been linked to human mitochondrial diseases with a broad range of clinical manifestations (Fuchs 2019). Recently, biallelic pathogenic variants in the *WARS2* gene (OMIM# 604733), encoding the tryptophanyl mt-aRSs (mtTrpRS), have been reported in 16 individuals with neonatal or infantile-onset disease presenting with phenotypes ranging from intellectual disability (Musante 2017), to parkinsonism (Martinelli, Burke, Virdee), leukoencephalopathy (Theisen, Maffezini), complex hyperkinetic disorder (Hubers) and neurodevelopmental disorder with abnormal movements, lactic acidosis with or without seizures (NEMMLAS) (Wortmann).

Here, we present additional 6 cases, expanding the spectrum of *WARS2*-related disease to later onset phenotypes including a more classic early-onset levodopa-responsive Parkinson's disease (PD) with abnormal dopaminergic imaging and a myoclonus dystonia phenotype.

Methods

Patients

Whole-exome sequenced (WES) subjects from collaborating movement disorder centers in Czech republic, Germany, Slovakia, and Switzerland were recruited within an ongoing research project aimed at identification of dystonia-related genes (families 1 and 2) (Zech 2020) or clinical genetic testing (families 3 and 4). Clinical information and genomic data were obtained after signing a written informed consent prior to enrollment in all subject. The study was performed according to the Declaration of Helsinki of 1975 and under standard protocols approved by local ethics committees.

Genomic testing

WES on genomic DNA isolated from blood lymphocytes was carried out using previously reported methods (Zech 2020). In short, exome capture was performed using Agilent SureSelect v5 or v6 kits and sequencing on Illumina HiSeq4000 or NovaSeq6000 systems was done with 100-bp paired-end reads at an average depth of coverage of > 100x. Sequence-data analysis and interpretation was performed at the Helmholtz Center Munich and Technical University of Munich (Munich, Germany) using a validated in-house-developed bioinformatics pipeline, as described (Zech 2020). In family 1, we sequenced the exomes of 2 affected siblings and the unaffected father; in family 2, quartet WES (2 affected siblings plus unaffected parents) was performed; in family 3, trio WES was performed (index patient plus unaffected parents); and in family 4, only the index patient underwent WES. The affected individuals` entire WES data sets were searched for pathogenic or likely pathogenic variants (Richards 2015) in reported disorder-associated genes, as detailed earlier (Zech 2020). Sanger sequencing was carried out to test for a biallelic status of identified *WARS2* variants in the index patient from family 4.

Functional studies

Patient-derived skin fibroblasts were expanded in culture, and the expression of the WARS2 protein in cell lysates was investigated using the western blotting method. Briefly, Fibroblast cell lines were expanded in growth medium (DMEM, 15% FCS, penicillin, streptavidin), and at 90% confluence lysed in RIPA lysis buffer (150 mM NaCl, 1.0% IGEPAL® CA-630, 0.5% sodium deoxycholate, 0.1% SDS, and 50 mM Tris pH 8.0.) containing protease inhibitor Complete® (Roche, Basel, Switzerland). Lysates were separated on 4-15% Criterion TGX precast gels (Bio-Rad Laboratories, Hercules, California), and transferred to nitrocellulose using the Trans-Blot® Turbo™ transfer system (Bio-Rad). Blots were blocked using 5% Protifar (Nutricia, Amsterdam, The Netherlands) in PBS, 0.1% v/v TWEEN® 20 (Sigma-Aldrich, St. Louis, Missouri). Primary antibodies used were: rabbit anti-WARS2 (Merck, HPA069692; 1:1000); rabbit anti-HSP60 (Cell Signaling Technology, 12165**,** 1:1000); mouse anti-Vinculin (Santa Cruz Biotechnology, sc-59803; 1:5000). After washing in PBS, 0.1% v/v TWEEN® 20, blots were incubated with fluorescently conjugated goat anti-mouse (IRDye 800) and goat anti-rabbit (IRDye 680) secondary antibodies (LI-COR Biosciences, Lincoln, Nebraska). After washing in PBS, 0.1% v/v TWEEN® 20, the blots were imaged and analyzed using an Odyssey imaging system (LI-COR).

Results

A total of 6 individuals from 4 families with biallelic variants in *WARS2* gene were identified (see Fig 1). Mean age of the individuals was 23.8±10.9 years, 4 were male and mean age of onset was 5.2±5.1 years (range 0-12 years). Detailed clinical characteristics of our sample along with a review of previously reported cases are presented in Table 1.

Family 1, Case P1 is a 36-year old male of Slovakian descent born after uneventful pregnancy and delivery. His first symptoms started at the age of 12 years with bilateral action hand tremor, which progressed to bilateral resting tremor at the age of 18 years and bradykinesia with rigidity at the age of 20 years. His symptoms gradually progressed until the age of 27 years when he was referred to a movement disorders specialist and was diagnosed with early-onset tremor-dominant PD with a correlate of abnormal DaT scan (see Fig. 2A), his MRI was repeatedly normal. He was initially treated with good effect of rotigotine on bradykinesia and rigidity, tremor was improved only partially and thus levodopa was added also with additional partial improvement of his tremor, which finally required adding propranolol to achieve acceptable compensation. Over the follow-up, the patient presents with prominent psychiatric features including major depression (BDI-II 28pts), anxiety, apathy and occasional irritability with outbursts of rage associated with non-complience to medications and psychotic symptoms requiring repeated psychiatric hospitalizations. Rotigotine has been discontinued and the patient is now well-compensated in terms of psychotic symptoms and irritability on a combination of clozapine and quetiapine. Currently he presents with prominent resting and postural jerky hand tremor, mild hypokinesia, no axial symptoms or freezing, occasional mild dyskinesia, mild cognitive impairment (MoCA 21pts), depression, anxiety, apathy, mild urine retention and paroxysmal sinus tachycardia (Suppl. video 1). A next-generation sequencing based dystonia panel performed at age 32 years did not identify a known genetic cause at that time.

Commented [MŠ1]: Marina - we have examined this patient together with your dystonia panel in Groningen back in 2016 if I remember correctly – please reference, correct or specify here as appropriate...

Family 1, Case P2, younger sister of subject P1, is a 34-year old female of Slovakian descent. Her first symptoms started at the age of 10 years with asymmetric jerky action hand tremor. At the age of 17 years, she developed cervical dystonia which required botulinum toxin A injections with good clinical effect. At the time of first evaluation in our center (age 26 years) she presented with jerky dystonic action hand tremor with a mild resting component and mild cervical dystonia, however, without rigidity and bradykinesia (Suppl. video 2). On sonography she had hyperechogenicity of substantia nigra. At the age of 32 years, she developed rigidity and bradykinesia satisfying the MDS clinical

criteria for PD, her DaT scan was abnormal in this period (see Fig. 1B), while her brain MRI has been repeatedly normal. Over the last 7 years, she also presents with gradually worsening prominent neuropsychiatric features, specifically severe anxiety disorder, including persistent and episodic component, and major depression (BDI-II 24pts). She recently presented also with visual illusions requiring neuroleptic treatment. Currently her motor features except for tremor are well compensated without any motor fluctuations on a combination of levodopa/carbidopa/entacapone and rasagiline, she presents with prominent neuropsychiatric features, with psychotic symptoms well compensated on quetiapine, but anxiety and depression being persistent, her cognition is normal (MoCA 28pts) (Suppl. video 2). She also complains of insomnia and constipation. Features indicative of atypical complex parkinsonism (Morales-Briceno 2020) were not present in any of the siblings from family 1.

Family 2, Case P4 is a 24-years old male of Czech origin. From 3 years of age he presents with a slowly progressive myoclonus of both upper and lower extremities and head as a dominating feature, very mild ataxia, mental retardation, mild cervical dystonia, intermittent axial dystonia and dystonic posturing of his upper extremities with a right-sided preponderance, more pronounced since the age of 11 years. On neurological examination he also showed slowed saccades, spasticity of lower extremities, social phobia (he is relatively well compensated on SSRI and cognitive behavioral therapy), mild to moderate mental retardation (Suppl. video 3). On paraclinical examinations screening for metabolic disorders was unrevealing, he had normal alpha-fetoprotein, normal brain MRI as well as liver and abdominal sonography.

Commented [PsW2]: Irena - both horizontal and vertical or just 1 specific plane?

Family 2, Case P3 is a 28-years old female of Czech origin with a milder manifestation and progression of the same disease as in her younger brother since the age of 6 years - abnormal movements of hands more pronounced on the left side which she calls “tremor” but clinically present a combination of myoclonic jerks and dystonia. She now presents with myoclonus, very mild hand ataxia, dystonia of her hands with a left sided predominance and mild cervical dystonia (Suppl. video 4). Her cognitive capacity is decreased but much less than in her brother, she managed to finish primary school with assistance and later was trained to become a gardener.

Commented [MŠ3]: Irena did you receive results of her neuropsychological exam?

Family 3, Case P5 is a 14-year old male of Swiss origin, who was born after uneventful pregnancy and delivery and had a normal early development in the first year of his life. From 18 months of age he developed myoclonus, reported by his parents as an „uncontrollable shaking“ during excitement, fever, etc., which progressed to continuous myoclonus by the age of 2 years. Metabolic testing and brain MRI were unrevealing at this age. Subsequently, at the age of 3 years he also developed action tremor, limb hypertonia and Babinski sign and subsequently dystonic and choreic involuntary movements. His cognition was reportedly normal, although there was a clear speech developmental delay, especially in the expressive language skills domain. By the age of 6-8 years the disease has significantly progressed in terms of dysarthria and dysphagia requiring PEG placement, the patient was wheelchair-bound. By the age of 11 years his weight, height and head circumference were below 3rd percentile, his clinical finding was dominated by spasticity more pronounced on upper limbs, generalized dystonia, chorea and myoclonus. His EEG was normal, he did not experience significant therapeutic improvement from a combination of tetrabenazine, L-dopa, gabapentine and baclofen.

Commented [MŠ4]: Robert could you please specify on the effect of pramipexole here?

Family 4, Case P6 is an 8-year old male of German descent, who was born by acute section in the 35th week of pregnancy. Problems with weight gain and unstable body temperature were noted in the postnatal phase. His developmental milestones including speech as well as gross and fine motor skills were slightly delayed. He currently presents with a combination of mild speech problems, mild ataxia and dominating action hand tremor which is intensified with emotional excitement and physical

exertion. After prolonged exercise like swimming, he seems to have less strength in his right leg based on history from parents, although no weakness or pyramidal signs are present on objective examination. His recent EEG was pathologic due to polyspikes bifrontally and irregular generalized spike-wave paroxysms up to 2-3 seconds without clinical correlation, there are no reported clinical seizures in his history. Otherwise, brain MRI, ophtalmological, endocrinological examinations and metabolic screening were unremarkable.

Commented [MŠ5]: Laura - do you think this is paroxysmal weakness or some sort of paroxysmal dystonia/dyskinesia?

Genetic studies

WES identified compound heterozygous *WARS2* variants in all affected subjects. Each of the identified *WARS2* sequence changes qualified as a "pathogenic" variant according to American College of Medical Genetics & Genomics guidelines (Richards 2015). A relatively common missense variant, c.37T>G, p.(Trp13Gly), for which a disease-causing effect has been demonstrated in several previous reports (Musante, Martinelli, Hubers, Burke), was shared by all 6 affected subjects. This variant occurred in compound heterozygosity with a previously described exon 2-deletion in both affected siblings from family 1; a previously described c.298_300delCTT, p.(Leu100del) variant predicted to result in the deletion of a highly conserved amino acid in both affected siblings from family 2; a previously described c.149G>A, p.(Gly50Asp) missense variant predicted to result in the substitution of a highly conserved amino acid in the index patient from family 3; and a novel c.622G>T, p.Glu208* nonsense variant predicted to result in premature termination of protein translation in the index patient from family 4.

Expression studies

To determine the effects of the variants observed in patients P1 and P2 (c.37T>G, p.(Trp13Gly); deletion exon 2) on WARS2 protein expression, western blotting analysis was performed. These data demonstrate markedly lower levels of full-length WARS2 protein in fibroblasts from patients P1 and P2 when compared to three unrelated controls (Fig. 3).

Discussion

Here we present clinical, genetic and expression studies of 6 subjects from 4 families having *WARS2*-related disease and expand the phenotypic spectrum of this disorder to later-onset phenotypes presenting with early-onset levodopa-responsive PD with abnormal dopaminergic imaging and to a myoclonus dystonia phenotype.

Using unbiased whole-exome sequencing approach we identified 6 subjects carrying compound heterozygous variants in *WARS2* gene. The p.Trp13Gly variant found in all our affected subjects is a relatively common variant, present in 922 heterozygotes and 6 homozygotes in GnomAD. Nevertheless, this variant has been confirmed as pathogenic in several previous reports (Burke, Musante, Hubers, Martinelli) and was shown to impact correct localization of WARS2 protein in cells (Musante) and variably affect the OXPHOS system (Virdee). Cumulative evidence therefore shows, that the p.Trp13Gly is a hypomorphic allele, causing disease in trans with loss-of-function mutations; likely associated with milder disease phenotypes and later disease onset as shown in several of our subjects.

A larger 36kb deletion including deletion of exon 2, found in subjects P1 and P2, was previously described in a single subject of Slovakian descent (Wortmann), similar to the origin of our patients. Their subject presented with a severe fatal neonatal form of *WARS2*-related disease, what is in contrast to a rather late (10-12y) onset of disease in our cases. This may be hypothetically explained by presence of another variant with a higher pathogenicity and eventually other modifiers in their case in contrast to presence of the p.Trp13Gly variant with a rather mild pathogenicity in our subjects.

The in-frame deletion c.298_300delCTT, p.(Leu100del), identified in subjects P3 and P4, results in deletion of a highly conserved amino acid (Theisen) which is located in the tryptophanyl-tRNA synthetase catalytic core domain (TrpRS core), described previously in a case with infantile onset leukoencephalopathy. In contrast to the previously described case, MRI in all our subjects was unremarkable, without signs of white matter abnormalities.

The c.149G>A, p.(Gly50Asp) missense variant which was not found in any of the control databases, was also previously described in a subject with an identical genotype as found in our subject P5 - p.Trp13Gly, p.Gly50Asp (Hubers). The phenotype of the subjects is rather similar, dominated by hyperkinetic movement disorders, including chorea and dystonia, stepwise loss of already acquired skills and age of onset around 15-18 months. Additionally, our case initially presented with myoclonus, which was observed throughout his disease course and later developed a severe spastic phenotype eventually leading to a wheelchair bound state and dysphagia requiring PEG placement.

The c.622G>T, p.Glu208* nonsense variant has not been described previously and it was absent from all examined control databases. This sequence alteration is predicted to result in a stop-codon located in exon 5 leading to a truncated and non-functional WARS2 protein. Similar to several of the previously described cases, this subject had a neonatal onset of symptoms and delay in developmental milestones, the finding was dominated by speech problems, ataxia, tremor and paroxysmal exercise-induced leg weakness. While seizures were reported in several previous cases of *WARS2*-related disease (Wortmann, Maffezini, Martinelli), clinical seizures were not reported in relation to the pathological EEG findings in subject P6.

Commented [MŠ6]: Laura - this is an interesting finding – do you think it is really weakness as reported by parents or it is some sort of paroxysmal exercise-induced dystonia/dyskinesia? Do we have a video of this?

Enzymatic studies

Western blotting analyses in patient-derived fibroblasts, show markedly decreased expression of the full-length WARS2 protein in both compound heterozygous subjects (P1 and P2) carrying the p.Trp13Gly variant and deletion of exon2. This finding is consistent with previously reported data showing that the p.Trp13Gly variant, which is located within the mitochondrial targeting sequence (MTS), leads to reduced protein expression and impaired mitochondrial localization (Martinelli, Burke, Musante). These findings further delineate these WARS2 variants as loss-of-function mutations.

Three cases with infantile onset parkinsonism caused by bialelic *WARS2* mutations have been described previously, all presenting also with action limb tremor, treatment-induced dyskinesia in 2 subjects (Martinelli, Burke) and seizures along with oculogyric crisis, ptosis, supranuclear gaze palsy, exotropia and severe derangement of DaT scan in one of the patients (Martinelli). In contrast to the previous reports, subjects P1 and P2 presented with a significantly later onset of parkinsonism, starting uniformly as action hand tremor around age 10-12 years, later accompanied by cervical dystonia in subject P2. Diagnosis of PD was made at the age of 27 years in case P1 and 32 years in case P2. Differential diagnosis of early onset PD is broad. Our patients presented with a slowly progressive parkinsonism, without features suggestive of atypical complex parkinsonism and their presentation is largely overlapping with phenotypes of the classical early-onset PD genes with

autosomal recessive inheritance, including Parkin, DJ1 and PINK1 (Kasten 2018, Morales-Briceno 2020). Although their parkinsonism is well responsive to dopaminergic medication, a jerky dystonic hand tremor is persistent and dominates the motor features of their disease. Dopamine agonists had to be discontinued in both cases due to psychotic complications and their non-motor phenotype is dominated by prominent neuropsychiatric symptoms, including anxiety and depression. Cognition is normal in the younger sister, what is in line with the general observations in Parkin, DJ1 and PINK1 carriers (Kasten 2018), while it is in the range of mild cognitive impairment (level 1) in her older brother. Abnormal DaT scan in both siblings points to a neurodegenerative background with nigrostriatal denervation.

Myoclonus, not previously described in relation to *WARS2* gene mutations, was a presenting feature in our cases P3, P4 and P5 and a myoclonus-dystonia (M-D)-like syndrome was the dominating phenotype in cases P3 and P4, expanding the spectrum of *WARS2*-related disease. Approximately one third of familial M-D are caused by SGCE mutations (Roze 2018). In SGCE-negative M-D, several other rare causes, including the *KCTD17*, *ADCY5*, *TITF1*, *ANO3*, *RELN*, *SCN8A*, *CACNA1B*, or *KCNN2* mutations, have been recently described and a significant proportion of cases still awaits elucidation of their etiologic background (Roze 2018, Balint 2020). The phenotype of subject P3 with relatively pure myoclonus dystonia present in neck and upper limbs and age of onset of 6 years is especially resemblant of *SGCE*-related M-D, although effect of alcohol was not tried and some mild cognitive problems are present. The phenotype in subject P4 is more complex and lower limbs involvement including spasticity along with mental retardation and slow ocular saccades is rather different from the *SGCE* M-D phenotype and would be more compatible with presentations of other M-D related genes, such as the ADCY5 (Vijiaratnam 2019).

In conclusion we present 6 additional cases with bialelic *WARS2* mutations and expand the spectrum of the disease to later onset new phenotypes of levodopa-reponsive early-onset tremor-dominant PD with abnormal dopaminergic imaging and prominent neuropsychiatric features and myoclonus dystonia phenotype. The role of *WARS2* mutations in the above-mentioned phenotypes needs further elucidation, nevertheless, in case of suspected *WARS2*-related disease, relaxing of exome filters might be crucial in order to prioritize the recurrent causative p.Trp13Gly variant found in all of our cases. While no specific therapy has been suggested so far, Virdee et al. (2019) reported a possible therapeutic benefit of administration of a mitochondrial cocktail, including Coenzyme Q10, vitamin E, a-lipoic acid and creatine monohydrate. In addition, interventions targeting mitochondrial function alteration, such as exenetide (Athauda 2017), may be an option for consideration in *WARS2*-related parkinsonism in the future.

Commented [MŠ7]: For consideration whether we want to expand a little also on the therapeutic options or we leave it out and keep only as a clinical/genetic paper without treatment considerations...?

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Commented [MŠ8]: Any additional local fundings or corrections?

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Figure Legends:

Fig 1 Genetic and clinical characteristics A) Pedigrees of included families with biallelic variants in WARS2; B) Location of disease-causing variants on the WARS2 protein scheme. Previously reported variants are reported above the cartoon, currently identified variants are reported below the cartoon, dots indicate number of reported families with a given variant. The mitochondrial targeting sequence and the aminoacyl-tRNA synthetase conserved site are shown in yellow and red, respectively.

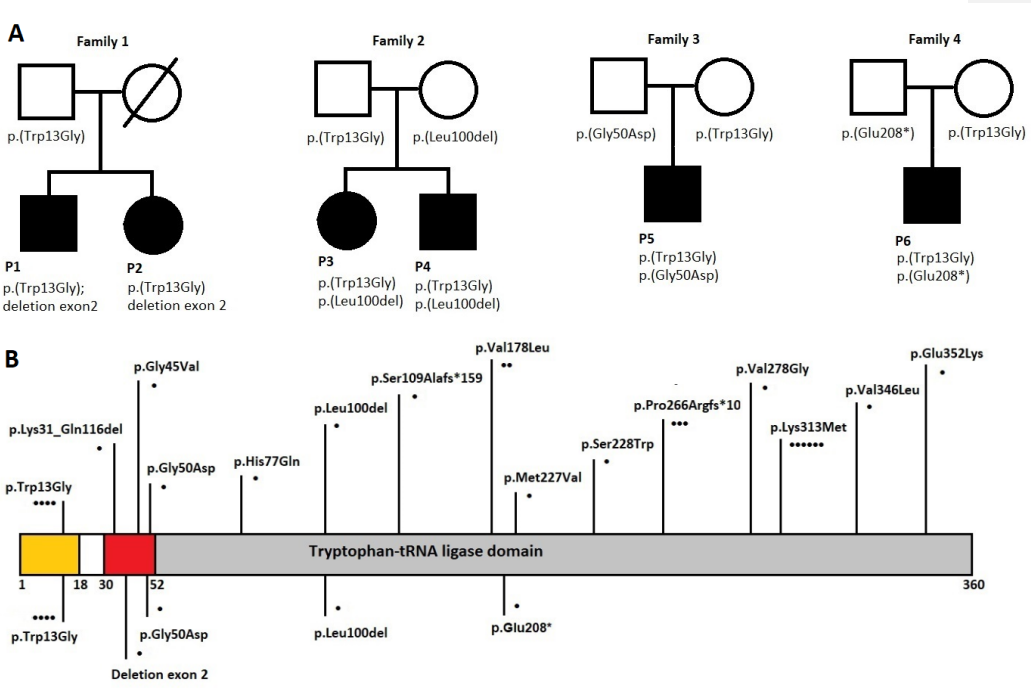

Figure 2 DaT scans in subjects P1 and P2 presenting with early-onset PD: A) reduced radiotracer uptake on DaT scan of subject P1 at the age of 27 years; B) reduced radiotracer uptake on DaT scan of subject P2 at the age of 32 years

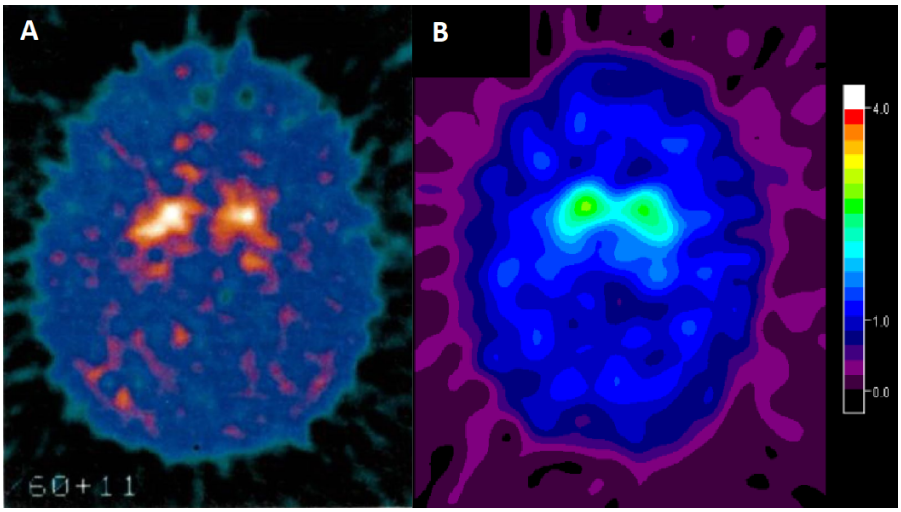

Fig. 3. **A.** Representative western blot analyses of fibroblast derived protein extracts from individuals with WARS2 mutations (compound heterozygous p.Trp13Gly variant; deletion of exon2)(P1, P2) and unrelated controls (Control 1-3). Vinculin and HSP60 are shown as loading controls. Molecular weights (kDa) are indicated. **B, C.** Quantitative analysis of WARS2 protein levels in subject's P1 and P2 fibroblasts, and 3 unrelated controls after normalization to Vinculin (B) or HSP60 (C). Data are expressed as mean ± SD (*: p≤0.05, **: p≤0.01, Student's t-test.)